BUD SPROUTING AND GROWTH OF *CYPERUS ROTUNDUS* L. ALTERED BY CYTOKININS

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ABSTRACT

Varying concentrations of N-6-benzyl adenine (BA), indoleacetic acid (IAA), gibberellic acid (GA), abscisic acid (ABA), and 2-chloroethylphosphonic acid (ethephon) were used to induce sprouting of dormant nutsedge (Cyperus rotundus L.) tubers. BA at 50 to 300 ppm stimulated sprouting. The continuous presence of BA during the sprouting period was necessary to give significant sprout stimulation. Kinetin and 6-benzylamino-9-(tetrahydropyran-2-yl)9H-purine (PBA) showed similar stimulatory effects on sprouting. Neither IAA at 1, 10, or 100 ppm; GA at 10, 100, or 1000 ppm; nor ethephon at 10, 100, or 1000 ppm had stimulatory effects on sprouting. ABA reversed the stimulatory effects of BA when tubers were treated with ABA following BA treatment. Sprouting was markedly greater at 33 C day, 25 C night than at 24 C day, 17 C night. There was no difference between cytokinin-induced sprouting in single tubers and that of tubers in intact rhizome chains. Enhanced sprouting was the same in light or darkness. Soil-applied cytokinin resulted in sprout stimulation similar to that observed in petri dishes and in sand culture.

Growth of plants originating from tubers pretreated with 100 ppm BA did not differ significantly from the controls. Sustained foliar applications of BA at 100 and 200 ppm produced numerous plants with tuft-type growth habit,

delayed flowering, and reduced the number of inflorescences. Numerous short, diageotropic rhizomes were produced.

The acidic ether extract from purple nutsedge tubers showed the presence of inhibitory substances in the acidic ether fraction. These inhibitors were generally referred to as inhibitor β. Inhibitor β inhibited sprouting of excised purple nutsedge buds as well as elongation of wheat coleoptile. The inhibition of bud sprouting by inhibitor β was relieved by BA. Application of ABA also inhibited sprouting of the excised buds and this was similarly reversed by BA applications. Inhibitor β mainly consisted of phenols and possibly ABA as a minor component.

The suggested role of BA in enhancing sprouting of purple nutsedge tubers was to antagonize inhibitor actions. The feasibility of the use of cytokinin-like substance to precondition purple nutsedge for subsequent eradication was discussed.

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CHAPTER I

INTRODUCTION

Purple nutsedge (Cyperus rotundus L.) has been described for some 50 years as a "formidable weed of cultivation" (82). The dormancy exhibited by the reproductive structures (tubers and basal bulbs) of purple nutsedge is perhaps the most important single characteristic enabling it to persist and flourish. These propagative structures have numerous dormant buds that are capable of sprouting after the foliage has been killed by applied post-emergence herbicides (39). Complete eradication of this weed is therefore seldom attained in cropland. A possible approach to effective eradication of this weed is to stimulate all the dormant buds on the tuber to sprout, thus exposing them to subsequent herbicidal treatment. After the tubers are exhausted of their viable buds, no rejuvenation of shoots is expected.

The understanding of bud dormancy with possible enhancement of tuber sprouting is a prelude to devising such an eradication method. The objectives of this study were: (1) to enhance sprouting of purple nutsedge tubers with the use of a growth substance, (2) on the success of the first goal, to study why such a substance is effective in releasing dormancy in relation to the possible causes of bud dormancy.

CHAPTER II

REVIEW OF LITERATURE

Purple nutsedge (Cyperus rotundus L.) is a perennial weed that infests agricultural lands world-wide. It is distributed throughout the warm regions of the world (28, 34). According to a survey by Holm and Herberger (34) purple nutsedge is considered the world's worst weed.

Biology of Purple Nutsedge

Since purple nutsedge produces few viable seeds (42), the principal method of propagation is through the basal bulb and tuber. The basal bulb is a corm which forms the base of the plant. Aerial leaves arise one-third phyllotaxy from the nodes of this corm. Buds are formed in the axils of the leaves. The tuber is an oval or spherical compressed stem with three to ten buds distributed spirally at the nodes. Deciduous scale leaves cover the buds. The exterior of the tuber is at first white but it changes color with age through brown to black (62). Both the basal bulb and the tuber serve as food storage organs. These structures originate from the meristematic cells of the rhizome apex (89).

A young rhizome is at first white and succulent but with age it becomes black and wiry. Anatomical and translocation studies of the old rhizome showed that it is completely intact and has a functional vascular system (2,

37, 63, 89). Observations on rhizome growth from a tuber grown in a box with slanting glass sides (53) showed the following development sequence:

(i) growth of bud from the tuber forming a rhizome.

(ii) cessation of longitudinal growth after growth for some length.

(iii) formation of a tuber at the region 2 to 3 mm back of the rhizome apex.

(iv) renewal of rhizome growth from the newly developed tuber.

Ranade and Burns (62) recognized three types of rhizomes: (i) the positively geotropic rhizomes or the "droppers" which grew into the deeper soil layer, (ii) the negatively geotropic rhizomes which grew to the soil level for light, (iii) the diageotropic rhizomes which grew in a horizontal direction. The view that growth hormones participated in the control of rhizome orientation was reviewed by Phillips (60). A study carried out by the author showed that gibberellic acid (GA) and combinations of GA and N-6-benzyl adenine (BA) evoked erect rhizome growth in purple nutsedge. Similar erect rhizome growth was observed in yellow nutsedge with GA treatment (7).

Under field conditions early growth of purple nutsedge consists of a rhizome emerging from the tuber. This rhizome terminates in above-ground foliar parts with a basal bulb below the soil surface (25). The new basal bulb

gives rise to more rhizomes, which after elongation for varying distances terminates in more basal bulbs or tubers. Thus in an established purple nutsedge stand, a complex interconnected system of plants, rhizomes, basal buls, and tubers is present. In an experiment in Georgia, tubers planted at 93 cm intervals produced 2.32 million plants and 2.76 million tubers and basal bulbs per acre after one season's growth (24). These data clearly emphasize the reproductive capacity and seriousness of purple nutsedge as a weed. Purple nutsedge growing in the alkaline heavy clay soil of the Gezira (4) produced 90% of the tubers within the top 15.5 cm of the soil layer, and none below 31 cm. In Puerto Rico (53) 60% of the tubers were found within the top 7.7 cm of the soil, and another 25% occurred between 7.7 to 15.5 cm. Tuber distribution on Norfolk sandy loam in Alabama (70) showed a similar trend where a majority of the tubers occurred in the upper 15.5 cm of the soil and none deeper than 38 cm.

Two types of dormancy have been described in purple nutsedge. Tuber apical dominance (62, 70) was observed in isolated tubers where the apical bud usually sprouted first while other buds remained inhibited. These inhibited buds resumed growth when the foliage of the existing plant was killed (39). Dissection of tubers also caused sprouting of buds near the cut surface (53). The interconnected tuber-rhizome system also exhibited apical dominance just as an

individual tuber (70). When the intact tuber-rhizome chain was planted vertically, sprouting usually occurred only in the uppermost tuber. When the rhizome was severed or killed with heat all the tubers on the chain sprouted at equal rates (53). The elimination of this type of apical dominance explains in part why cultivation frequently appears to increase nutsedge infestation of an area (62, 70).

The cause of dormancy in purple nutsedge has not been established, though many workers suspect it to be due to the presence of growth inhibitors. In attempting to identify the inhibitors, Berger and Day (10) cited salicylic acid as the major component among the many inhibitors found in the foliage of purple nutsedge. Salicylic acid was suggested to cause dormancy in purple nutsedge even though it was not found in the tubers. Jangaard et. al. (39) thought ABA might be the inhibitor associated with nutsedge dormancy. They were not, however, able to detect the presence of ABA in the dormant tubers (personal communication). Jackson et. al. (38) reported that ethylene and ethephon stimulated bud sprouting in nutsedge. No information was available on the effects of other growth regulators on sprouting of nutsedge tubers.

Control of Purple Nutsedge

In 1925 Ranade and Burns (62) reported that control of this weed in India could be achieved by two successive

plowings during the hot season. Similar success was later reported by other workers (4, 71). Deep plowing of the soil to expose the tubers to the sun was essential to kill the propagative structures; tubers were readily killed through desiccation. Hollingworth and Ennis Jr. (33) noted that cultivation alone gave as good or better control than the use of herbicides. Thus, where practical, deep tillage seems to be an economical control method.

Herbicides like 2,4-D, amitrole, MSMA, dichlobenil, the substituted uracils, and the thiocarbamates were reported to give varying degrees of control (43). Two applications of amitrole were effective on nutsedge clones from a single tuber, but for an established stand, due to the varied growth stage, success was limited (26). Nutsedge was most susceptible to applied amitrole 4 weeks after initial emergence (27). Repeated applications (4 to 8 times/year) of MSMA at 5.6 to 16.8 kg/ha destroyed most established space-planted purple nutsedge in Arizona (23). Dichlobenil or terbacil at 6.7 to 9 kg/ha when incorporated in the soil gave excellent control for 12 to 18 months. These herbicides were highly persistent in the soil and enough remained in the soil for as long as 24 months to be toxic for subsequent crop growth (86). The thiocarbamates may be the most effective groups of compounds for nutsedge control. Herbicides included in this group are: EPTC, butylate and vernolate (5, 43). Soil incorporation of EPTC (3.7 kg/ha)

gave good seasonal control of purple nutsedge in Western USA. At such a rate EPTC was reported to cause bud inhibition of the tuber (5). However, the tubers were reported to be killed when exposed to soil-incorporated EPTC at 13.4 to 17.9 kg/ha for 8 to 12 weeks (36).

It is apparent from the various reports that no economical and effective eradication method for this weed is yet available. It seems that the main cause of this failure is the inability of applied chemicals to reach the underground reproductive structures which soon rejuvenate into shoots upon death of the existing foliage.

Physiology of Dormancy

Dormancy or rest is defined as the state in which the growth of a plant organ is in some way prevented even though the external conditions are apparently favorable. This phenomenon suggests the presence of an internal block (83) of growth processes. The onset of dormancy, its control and termination are apparently regulated by a balance of growth promoters and inhibitors (3, 81, 86). At its onset, this promoter-inhibitor balance is shifted in favor of the inhibitor components. This condition may be the result of either an excessive level of endogenous inhibitory substances or the absence or deficiency of the promoters. At the termination of dormancy the promoter-inhibitor balance is shifted back in favor of the promoters (81).

The endogenous promoters may include the gibberellins and the cytokinins. GA has been shown to break dormancy in seeds of lettuce (Lactuca sativa var. Grand Rapids) (11, 46), peaches (Prunus persica) (17), black currant (Ribes nigrum) (52), and various other plant organs (74, 84). However, the treatment of plant tissue with GA did not always cause the termination of its dormancy. Some authors, on the contrary, indicated that application of GA induced dormancy in Begonia evansiana (57), yam (Dioscorea batatas) (56), grapes (Vitis vinifera) (87), and some woody plant species (13). Stimulation of lateral bud growth in intact whole plants of peas (Pisium sativum) and Helianthus spp. was achieved by direct applications of cytokinins to the buds (67). Similar bud stimulation was reported in grapes (89), peaches (81), apples (Pyrus malus cv. Monroe) (45), Chrysanthemum morifolium cv. Mrs. Roy (14), and roses (Rosa hybrida cv. Forever Yours) with cytokinin application. Heide (29) found cytokinin treatment of Bryophyllum diagremontianum leaves greatly increased the number of epiphyllum buds but at the same time inhibited root formation. Although both GA and cytokinins were effective in breaking dormancy, it was suggested that each hormone affected the growth-triggering mechanism differently (47, 81).

Growth inhibitors in plants are exceedingly numerous and their chemical structures correspondingly varied (44,

48). The hypothesis that inhibitors were involved in bud dormancy was first suggested by Hemberg in 1949. He reported that the acidic ether soluble fractions from potato (Solanum tuberosum) peelings (30) and ash (Fraxinus excelsior) buds (31) showed inhibitory activity. A distinct correlation between the inhibitor content and sprout inhibition in potato was later established, leading to the hypothesis that dormancy in potato was regulated by these inhibitors (32). In 1953, these inhibitors came to be known as inhibitor β complex after Bennet-Clark and Kefford (8). Inhibitor β is a rather loose nomenclature given to inhibitory zones lying somewhere between Rf 0.4 to 0.9 when extract is fractionated with paper chromatography in isopropanol-ammonia-water system (35, 77, 86). Occurrence of inhibitor β seems widespread and it has been related to both dormancy and correlation effects in plants (48). Components of inhibitor β are reported to include toxic fatty acids (9), abscisic acid (ABA) (35, 51, 66), and phenolic compounds such as cinnamic, ferulic, o- and p-coumaric acids (79, 80), and salicylic acid (35). It is evident from these reports by different authors that inhibitor β extracted from various sources might not be the same substance or complex (50).

Besides inhibitor β, other naturally-occurring inhibitors such as cyanides (41), naringenin (70), and prunin (19) have been isolated and were proposed to be implicated with the dormancy of plant organs.

The growth inhibitor that is now receiving major attention is ABA (1), formerly known as dormin or abscisin II (58, 64, 75). ABA has been detected in many types of plants including ferns, grasses, coconut, bean, potato, avacado, apple, peach and roses. It has been observed in leaves, stems, buds, tubers, rhizomes, fruits, pollen, seedcoat, endosperms, and embryo (51, 81). According to Milborrow (51) ABA was the major and most active component of inhibitor β responsible for dormancy in certain plant organs. Some authors, however, doubted that the growth retarding effect of this inhibitor complex was entirely due to the small amounts of the ABA present, but might be due to the cumulative effects of a number of inhibitory compounds within the complex (44, 83).

In spite of the numerous studies on growth inhibitors, their physiological roles in vivo still remain unclear. To date there have been no experiments which demonstrated the physiological effects of natural growth inhibitors in intact cells (44). Most experiments with inhibitors have been carried out with biological tests after isolation from plant tissues. The mere presence of inhibitory substances in plant tissue does not necessarily indicate that such a substance is important in dormancy (82). A dormancy-inducing substance might cause growth inhibition but not all growth inhibiting substances could function as dormancy regulators in plants. Thus the presence of inhibitors in plant tissue

is necessary but not sufficient evidence to implicate its control in dormancy. It is necessary at least to quantitatively relate inhibitor level with dormancy process as evidence of its participation in the process (48, 83). Unfortunately, in some plants even such correlation may not necessarily be valid. A peak accumulation of phenolic compounds and ABA sometimes existed in young green leaves, while in the old abscissing leaves the level of these substances were sometimes much lower (44). Corgan (in 81) showed that ABA in peach flower buds fluctuated during the season but did not disappear with the termination of dormancy. The greatest ABA activity occurred near full bloom.

The evaluation of inhibitory activity of plant extracts is accomplished by using growth inhibiting tests like the wheat coleoptile assay (18, 35, 55). The most suitable test would be one in which the same species served both as the source of the inhibitors as well as the test material. It would also be highly desirable if the induction or removal of dormancy were used as the criterion for evaluation rather than growth inhibition (82).

The isolation procedure of growth inhibitors itself imposed limitations upon proper evaluation and interpretation of the results obtained. Wareing and Sauders (85) noted that even with the most elaborate fractionation procedures, there was no guarantee that a particular growth substance had been completely separated from other compounds

which might interact with the bioassay used. As an example, the use of paper chromatography in isopropanol-ammonia-water gave poor separation from each other of ABA, naringenin, and several gibberellins.

The mechanism of action of growth inhibitors is not clear. Likewise the mechanism of action of plant hormones is still not exactly known. Possibly the role of hormones such as cytokinins and gibberellins is to neutralize the effects of inhibitors (11, 44, 46, 47, 49, 50, 58, 69). Sprouting of the potato eyes was shown to be inhibited by inhibitor β. This inhibition was reversed by the application of GA (12). Cytokinin was reported to reverse ABA-induced inhibition effects in lettuce seeds (11, 46, 68) and duckweed (58). However, quantitative restoration of the normal growth of duckweed by cytokinins was achieved only if the ABA concentration did not exceed a critical level (58).

Growth inhibitors seemed to inhibit various processes within the plant such as ATP formation, nucleic acid and protein synthesis (1, 44, 58). Inhibition of such processes depressed any form of growth which might be induced by auxins, GA or cytokinins (44, 48, 50).

CHAPTER III

CYTOKININ-ENHANCED SPROUTING OF PURPLE NUTSEDGE TUBERS

It has been suggested that a possible approach to effective eradication is to stimulate all the inhibited buds on the tuber to sprout. It is only after all the buds on the tuber have sprouted and formed shoots that application of a herbicide is effective in its eradication. The understanding of tuber dormancy is important and a prelude to devising such an eradication method.

In this study various growth substances were evaluated for sprout enhancement activity of purple nutsedge tubers. Factors affecting the efficiency of sprout enhancement were studied. These included the length of treatment time required to induce response, the effects of temperature, light, and various media (filter paper, sand and soil) on sprouting. The effects of tuber chain and other physiologically related promoter compounds were also studied. Possible side effects or modifications of plant growth arising from the use of a sprout promoter were studied under sand culture in growth chambers.

Materials and Methods

Experiments were conducted at the University of Hawaii and The Ohio State University. Tubers were dug from the

fields at Waimanalo Research Station, Hawaii. Tubers were mailed from Hawaii for the work at The Ohio State University. In compliance with quarantine regulations, all tubers shipped were first washed to remove the soil. After arrival they were stored at 3 C for a period of 2 to 3 weeks until use. Tubers used in the experiments at Hawaii were not washed. Only black, dormant tubers were used.

Effects of growth regulators on sprouting: Various concentrations of N-6-benzyl adenine (BA), indole acetic acid (IAA), gibberellic acid (GA,75% K salt) and ethephon were used. BA, IAA, and GA were obtained commercially from Nutritional Biochemicals Corp., and ethephon from Amchem Products.

Tubers were soaked for 6 hours in the growth regulator solutions before placing them in petri dishes on three pieces of Whatman No. 2 filter paper. Ten tubers were used for each treatment. The filter papers were moistened initially with 4.5 ml of the growth regulator solutions used for soaking the tubers. Distilled water was used for subsequent watering. A count of sprouts was made after 10 days. Treatments for all experiments were replicated 4 to 10 times. Sprouting was done in an ambient room environment. At Hawaii, the temperature was 24 $\pm$ 2 C, and at Ohio 30 $\pm$ 5 C. Three controls were used for the experiments. One control was water and ethanol, with ethanol at concentrations

similar to those of the BA solutions. Another control was water and HC1 to adjust the pH equivalent to that of the ethephon solutions used. These two controls would indicate if there were any effects of ethanol as solvent for BA or a pH effect when ethephon was used. The third control was distilled water only.

Effect of soaking time in BA solutions: Tubers were soaked in 100 ppm BA for 3, 6, 12, and 24 hours before sprouting. No BA was added to the sprouting medium, except in one treatment to show the effect of the continuous presence of BA.

Effect of abscisic acid (ABA) and combinations of BA and ABA: Tubers were first soaked in 1, 10, and 100 ppm ABA (Shell Development Co.,) for 6 hours and then left to sprout on filter paper in petri dishes. The filter paper was initially moistened with 4.5 ml of ABA solutions used for soaking the tubers. Sprouting was evaluated after 10 days.

Similar procedures were followed to study the effects of the combinations of BA and ABA on tuber sprouting. The solutions used were: 100 ppm BA + 1 ppm ABA; 100 ppm BA + 10 ppm ABA; and 100 ppm BA + 100 ppm ABA. After sprouting was evaluated at 10 days, the tubers were washed in distilled water and placed again in petri dishes to sprout on media provided with 100 ppm BA.

Effects of temperature: Tubers treated with 1, 10, 50, and 300 ppm BA were sprouted on filter paper provided with BA as described earlier. They were placed in growth chambers at 33 C day, 25 C night and 24 C day, 17 C night with 12-hour photoperiods.

Effects of tuber chain: Single tubers and intact tuber chains each consisting of four tubers were sprouted on filter paper provided with 100 ppm BA or water and sprouted as described earlier. In another treatment only one tuber within the intact chain was treated with 100 ppm BA while the remaining tubers were sprouted on water-moistened filter paper. This was achieved by placing a 3 cm petri dish with filter paper treated with BA, within a 12 cm petri dish in which the filter paper was moistened with water only. Sprouting was evaluated after 10 days.

Effects of light: Tubers were sprouted on filter paper provided with 100 ppm BA or water under continuous light or complete darkness at 24 $\pm$ 2 C. Sprouting was evaluated after 10 days.

Effects of sand-applied kinetin and BA: One tuber was planted in each wax cup filled with sand. The sand was watered with 50 ppm kinetin (Nutritional Biochemical Corp.) or BA once and subsequently half-strength Hoagland solution

was applied daily. Sprout count was made after 14 days. Temperature of the growth chamber was at 33 C day and 25 C night with 12-hour photoperiod.

Effects of soil-applied 6-benzylamino-9-(tetrahydropyran-2-yl)-9H-purine (PBA) and BA: Tubers were grown in soil in aluminum foil trays, each measuring 8 x 14 x 5 cm. The soil was mixed with perlite (10% v/v) to aid in aeration and porosity. The soil was initially drenched with PBA (Shell Development Co.) or BA solutions with concentrations of 0, 5, and 100 ppm at the rate of 125 ml per 350 g soil. Tubers were planted 2.5 cm deep. Shoots produced per tuber were counted after 12 days.

The sprouted tubers were dug out of the soil, washed and their sprouts removed. These tubers were then tested for their capacity for further sprouting by continuous exposure to 100 ppm BA in petri dishes. A count of the sprouts was made after 7 days. This study was conducted in a growth chamber at 30 C with a 12-hour photoperiod.

The duration of BA activity in soil was also studied. Tubers were planted in the soil 0, 1, and 2 weeks after the soil was treated with 100 ppm BA as described earlier. Shoots produced were counted after 7 days. This experiment was conducted in the greenhouse. The temperature was 30 $\pm$ 5C.

Effects of BA on plant growth: Plants were grown in the growth chamber at 33 C day, 25 C night with a 12-hour photoperiod. Three plants were planted in each 5-liter plastic pail filled with sand. Each of the three pails served as a replicate. Plants in the pails were watered automatically, as described by Bendixen (7), with half-strength Hoagland solution. Nutrient solutions were changed weekly during the course of the experiment.

Growth of pretreated tubers: Tubers were treated with 100 ppm BA and allowed to sprout for 10 days in petri dishes. Sprouted tubers were then planted three per pail as described above. The plants were harvested 39 days after planting and various growth parameters were noted, viz. numbers and lengths of rhizomes, number of plants, and number of inflorescences present. The original tubers were then removed and exposed to 100 ppm BA in petri dishes to test their capacity to produce additional sprouts. It was assumed that any remaining sprouts would develop following this treatment and that the total number of sprouts could then be estimated.

Growth of repeated foliarly-treated plants: Ten-day-old plants were excised from the tubers and planted three per pail. Three days later, they were treated with BA at 10, 100, and 200 ppm. Ten μL of BA was applied to the

youngest leaf of each plant daily throughout the course of the experiment. Tween 20 (Polyoxyethylene (20) sorbitan monolaurate at 0.05%) was added to the BA solutions. The plants were harvested after 36 days and the various growth parameters, as above, were noted.

Results

Effects of growth regulators on sprouting: BA at 50 and 300 ppm significantly stimulated sprouting and produced two to three times as many sprouts per treatment as the controls (Table 1). The effect of increased BA concentrations was also apparent on the sprouts formed. Increased BA concentrations inhibited root formation and decreased mean length of sprouts. The sprouts were more uniform on each tuber as concentrations of BA increased (Table 1). Usually only one long apical sprout was formed in the controls and in the treatments with BA at 1 and 10 ppm. Other sprouts on the tuber, if any, were short. The range of sprout length, as shown by the maximum and minimum values in Table 1, was wider in BA treatments at 50 ppm or lower.

Neither IAA at 1, 10, 100, or 1000 ppm; GA at 10, 100, or 1000 ppm; nor ethephon at 10, 100, or 1000 ppm had stimulatory effects on sprouting. IAA at 100 ppm induced profuse rooting with little or no shoot development. At 1 ppm there was good shoot and root formation. GA stimulated shoot elongation at 1000 ppm but this effect was diminished

at lower concentrations. No significant effects were observed with ethephon treatments. Sprouting was not significantly different among the three control treatments, i.e., water control, water and ethanol, and water and HC1. This indicated that there was no apparent effect of either ethanol, used as a solvent for BA, or the high acidity of the ethephon solutions. Sprout stimulation was, therefore, specifically a consequence of BA treatment.

TABLE 1.--Effects of various concentrations of BA on sprout number and sprout length of purple nutsedge tubers (Each treatment consisted of 10 tubers. Sprouting was done in petri dish at 30 ± 5 C.)

Treatment	ppm	Sprouts/ Treatment	Sprout Length (mm)		
			Maximum	Minimum	Mean
Water control		19.2 b*	67 k	3	22
Ethanol control		18.0 b	72 k	3	22
BA	1	8.7 a	76 k	4	27
BA	10	23.9 b	74 k	2	20
BA	50	34.3 c	68 k	3	19
BA	300	48.4 d	32 j	4	14

*Means within a column with same letter are not significantly different at P = 0.05 (Duncan's Multiple Range test).

No significant effect was indicated from soaking tubers in BA for various lengths of time as compared to the water control (Table 2). The treatment in which the tubers remained in BA gave about twice the number of sprouts as compared to other treatments.

TABLE 2.--Effects of soaking nutsedge tubers in 100 ppm BA for various durations on sprouting of purple nutsedge tubers (In the BA control the tubers were provided with BA during the 10 days of the study. Each treatment consisted of 10 tubers. Sprouting was done in petri dish at 30 ± 5 C.)

Hours in BA	Sprouts/Treatment
0 (water control)	14.8 a*
3	13.8 a
6	14.0 a
12	16.5 a
24	15.5 a
240 (BA control)	29.0 b

*Means with same letter are not significantly different at P = 0.05 (Duncan's Multiple Range test).

Effects of ABA, and combinations of BA and ABA: ABA at 1 and 10 ppm had no significant effect on sprouting (either by itself or in combination with BA); but at 100 ppm ABA or 100 ppm ABA + 100 ppm BA, sprouting of purple nutsedge tubers was inhibited (Table 3). Sprouting occurred when the 100 ppm ABA + 100 ppm BA-treated tubers were washed and then left to sprout in a medium containing 100 ppm BA. There was a twofold increase of sprouting due to the removal of ABA from the medium.

TABLE 3.--Effects of ABA and combinations of BA and ABA on sprouting of purple nutsedge tubers (Ten tubers were sprouted in each petri dish provided with BA, ABA, or a mixture of BA + ABA. After evaluation of sprouting at 10 days those tubers treated with BA + ABA were further exposed to BA to induce remaining buds to sprout. Sprouting was done at 30 ± 5 C.)

Treatments	ppm	Sprouts/Treatment			
		ABA	BA + ABA	BA + ABA ⟶ BA	Increase
BA control	100	-	22.3 a	22.3 a	-
Water control	-	10.3 de*	-	-	-
ABA	1	13.5 cde	18.5 abc	22.3 a	3.8
ABA	10	8.6 e	21.8 ab	24.8 a	3.0
ABA	100	0.3 f	8.3 e	15.5 bcd	7.2

*Means with the same letter are not significantly different at P = 0.05 (Duncan's Multiple Range test).

Effects of temperature: Temperature exerted a significant role in sprouting. High temperatures (33 C day, 25 C night) tended to increase sprouting (Table 4). BA-stimulated sprouting was not observed at the low temperature regime (24 C day, 17 C night), and overall sprouting was reduced tremendously in comparison with the high temperatures.

TABLE 4.--Effects of BA on sprouting of purple nutsedge tubers under two temperature regimes (Each treatment consisted of 10 tubers.)

Treatments	ppm	Sprouts/Treatment	
		33 C day 25 C night	24 C day 17 C night
Water control		23.6 a*	6.0 e
Ethanol control		22.8 a	3.8 e
BA	1	9.2 b	5.8 e
BA	10	20.6 a	5.0 e
BA	50	31.4 c	13.0 b
BA	300	46.4 d	6.0 e

*Means with the same letter are not significantly different at P = 0.05 (Duncan's Multiple Range test).

Effects of tuber chain: There was no significant difference between the number of BA-enhanced sprouts on single tubers and those in intact tuber chains (Table 5). When only

one tuber within the intact chain was treated with BA, while the remaining tubers were sprouted on water-moistened filter paper, only the BA-treated tuber had increased number of sprouts. The untreated tubers had sprouts not significantly different from the control (Table 6). Although rhizomes connecting the tubers were shown to be functional (2, 37, 63, 89), these results seemed to indicate that BA was not readily translocated from one tuber to induce sprouting at another. For enhanced sprouting, BA needs to be in contact with the tuber.

TABLE 5.--A comparison of the effects of BA on sprouting of single purple nutsedge tubers and those in intact tuber chains. (There were four tubers in each intact tuber chain. Sprouting was done in petri dishes at 30 ± 5 C.)

Treatment	Tuber	Sprouts/Tuber
Water control	single	0.8 a*
BA 100 ppm	single	4.8 b
Water control	chain	0.7 a
BA 100 ppm	chain	4.0 b

*Means with the same letters are not significantly different at P = 0.05 (Duncan's Multiple Range test).

TABLE 6.--Sprouting of purple nutsedge tubers in intact tuber chains when only one tuber in a chain was treated with BA (There were four tubers in each intact tuber chain. The tubers were numbered consecutively from the oldest to the youngest, i.e., No. 1 being the oldest in the chain. Sprouting was done in petri dishes at 30 ± 5 C.)

Tuber treated with BA 100 ppm	Sprouts/Tuber			
	Tuber Number			
	1	2	3	4
Tuber No. 1	5.3 a*	1.5 b	1.3 b	1.0 b
2	0.8 b	5.2 a	1.0 b	0.8 b
3	0.5 b	0.8 b	5.8 a	1.3 b
4	1.5 b	0.7 b	1.8 b	6.8 a

*Means with the same letters are not significantly different at P = 0.05 (Duncan's Multiple Range test).

Effects of light: Tubers sprouted in darkness formed rhizomes. Those sprouted under continuous light formed expanded green leaves, the base of which were swollen forming a basal bulb. Such structures formed in light are arbitrarily referred to here as plantlets. Buds on the tuber would form either rhizomes or plantlets depending on the availability of light. The effect of BA on tuber sprouting was the same in light or darkness (Table 7). With BA treatment rhizomes were short and each rhizome had three to four nodes regardless of its length. The apex had a swollen appearance and was covered with soft scale leaves.

In contrast, the control tubers produced longer rhizomes and there were four to seven nodes on each rhizome depending on its length. The rhizome apex consisted of pointed scale leaves. The scale leaves of the rhizome apex turned green and differentiated into leaves (i.e., forming plantlets) when the rhizomes were exposed to continuous low light intensity (40W, cool white flourescent, 1800 lux) for 2 to 3 days. Under light the plantlet formed very close to the tuber and no rhizome was evident externally. Dissection through the plantlet and the tuber revealed a rhizome-like structure consisting of a well-defined vascular core uniting the two structures.

TABLE 7.--Effects of BA on sprouting of purple nutsedge in darkness and light (Five tubers were placed in each petri dish. Plantlet refers to sprout with expanded green leaves, the base of which is swollen forming a basal bulb.)

Treatment	Structure		
	Form	Number/ Tuber	Length (mm)
Water control in darkness	Rhizome	1 a*	71
BA (100 ppm) in darkness	Rhizome	4 b	39 +
Water control in light	Plantlet	1.5 a	95
BA (100 ppm) in light	Plantlet	4.2 b	43 +

*Means with the same letter are not significantly different at P = 0.05 (Duncan's Multiple Range test).

+Significant from their respective controls at P = 0.05 (t test).

Effects of sand-applied kinetin and BA: The number of sprouts produced per tuber planted in sand was not significantly different between kinetin and BA and was five times more than the control. Both kinetin and BA produced sprouts about one-third shorter than the control and root formation was poor (Table 8).

TABLE 8.--Effects of kinetin and BA on sprout number, sprout length, and root formation of purple nutsedge tubers (One tuber was planted in each wax cup filled with sand. Sprouting was done in growth chamber at 33 C day and 25 C night.)

Treatment	Sprouts/ Tuber	Sprout Length (mm)	Root Formation
Water control	1.3 a*	147 j	Good
BA 50 ppm	5.7 b	57 k	Poor
Kinetin 50 ppm	6.2 b	41 k	Poor

*Means within a column with the same letter are not significantly different at P = 0.05 (Duncan's Multiple Range test).

Effects of soil-applied PBA and BA: Both PBA and BA at all concentrations tested (i.e., 50 and 100 ppm) significantly increased the number of shoots produced per tuber (Table 9). There was no significant difference in all parameters measured between the four PBA and BA treatments. Seventy-two to eighty-eight percent of the buds present on

the tuber sprouted when grown in the soil treated with either PBA or BA, while in the control only 25% of the buds sprouted (Table 9).

BA activity in the soil lasted for less than one week. There was no significant difference between sprouting of tubers planted 1 or 2 weeks after the soils were treated with 100 ppm BA and the controls (Table 10). This suggests breakdown or decomposition of BA in the soil.

TABLE 9.--Effects of soil-applied PBA and BA on sprouting of purple nutsedge tubers (Six tubers were planted in a tray with 350 g soil provided with 125 ml PBA or BA. After 12 days the shoots were removed and the tubers exposed to 100 ppm BA in petri dishes. The total number of buds present on the tuber was calculated assuming that the second BA treatment induced all buds on the tuber to sprout.)

Treatment	ppm	Sprouts/Tuber		Total	% Sprouted in Soil
		In Soil	In Petri Dish + 100 ppm BA		
Water control		1.3 a*	3.8	5.2	25 j
BA	50	4.3 b	1.7	6.0	72 k
BA	100	4.3 b	1.2	5.5	78 k
PBA	50	3.8 b	0.2	4.3	88 k
PBA	100	5.2 b	1.0	6.2	84 k

*Means within a column with same letter are not significantly different at P = 0.05 (Duncan's Multiple Range test).

TABLE 10.--BA activity in soil as indicated by its ability to enhance sprouting of purple nutsedge tubers (Tubers were planted 0, 1, and 2 weeks after the soil was treated with 100 ppm BA.)

Weeks After Application	Sprouts/Tuber	
	Untreated Soil	BA-Treated Soil
0	1.7 a*	4.1 b
1	0.9 a	1.6 a
2	1.2 a	1.8 a

*Means with same letter are not significantly different at P = 0.05 (Duncan's Multiple Range test).

Growth of pretreated tubers: Growth of plants from tubers pretreated for 10 days with 100 ppm BA did not differ significantly from those of the untreated tubers (control). The plants began to flower on the 16th day after planting (i.e., at 26 days old) and by the 18th day most plants had inflorescences. There was no significant difference in the time of flowering, the number of inflorescences, or the total number of plants produced per tuber between the BA pretreated tubers and the controls. The effects of BA on the number and total length of rhizomes were also not significantly different. When these tubers were subsequently exposed to 100 ppm BA, the tubers previously treated with BA produced one or no additional sprouts while the controls produced several additional sprouts per tuber (Table 11).

This indicated that the original BA treatment was effective in inducing most buds (89%) present on the tubers to sprout.

TABLE 11.--Effects of BA treatment on tuber sprouting for the same tubers before planting and after 39 days of growth

Treatment before Planting		Treatment after 39 Days		Total Sprouts	Sprouted at First Treatment (%)
BA Treatment (ppm)	Sprouts/ Tuber	BA Treatment (ppm)	Sprouts/ Tuber		
0	2.0	100	2.7	4.7	43
100	5.6*	100	0.7	6.3	89*

*Significant at P = 0.05 (t test).

Growth of repeated foliarly-treated plants: The effects of foliar-applied BA at 100 and 200 ppm on growth of purple nutsedge were significant (Table 12). BA induced a tuft-type growth, and the leaves were short and dark green. Flowering was delayed and the number of inflorescences produced was reduced. The total number of plants produced from an initial plant was increased. BA induced production of numerous, but short, diageotropic rhizomes. The total length of rhizomes did not differ significantly from the control. The effects of 10 ppm BA was not significant. In the control, rhizomes showed branching, but in BA at 100 and 200 ppm such branching was not observed.

TABLE 12.--Effects of repeated foliar application of BA on growth of purple nutsedge plant (Plants were grown under sand culture in growth chamber for 36 days. Each plant was treated with 10 μL BA solutions daily.)

BA Treatment (ppm)	Plants	Inflorescences	Rhizome		
			Number/ Initial Plant	Length (mm)	Mean Length of Primary Rhizome
0	11.2 a*	4.2 f	19.1 j	1346 t	47 y
10	9.0 a	2.6 f	14.9 j	861 t	66 y
100	17.7 b	0.2 h	36.6 k	1469 t	21 z
200	19.4 b	0.5 h	34.9 k	1115 t	24 z

*Means within a column with same letter are not significantly different at P = 0.05 (Duncan's Multiple Range test).

CHAPTER IV

REVERSAL OF INHIBITOR β AND ABSCISIC ACID-INDUCED BUD INHIBITION BY BENZYL ADENINE

Bud dormancy in nutsedge has been suspected by many workers as being due to the presence of inhibitors (10, 78). In attempting to identify the inhibitors, Berger and Day (10) reported that salicylic acid was the major component among the many inhibitors found in the foliage of purple nutsedge, but it was not found in the tubers. Jangaard et. al. (39) thought that ABA might be the inhibitor associated with nutsedge tuber dormancy, however, they were unable to detect the presence of ABA in the dormant tubers (personal communication).

The objective of this study was to demonstrate that growth inhibitors and promoters participate in the control of bud sprouting in purple nutsedge tubers. In the earlier experiments enhanced sprouting of purple nutsedge tubers was enhanced by cytokinin treatment, and this enhanced sprouting was counteracted by ABA. ABA also inhibited sprouting of the tubers. This study evaluated the activities of ABA and inhibitors from the purple nutsedge tubers and showed their interactions with cytokinin on sprouting of excised purple nutsedge buds. In theory, the inhibitors from the tubers should be able to inhibit sprouting of these excised buds, and BA should reverse this inhibition.

Partial identification of the inhibitors from the tubers was also attempted.

Materials and Methods

Extraction of tuber inhibitors: The extraction procedure was modified from methods described by Milborrow (51) and Holst (35). One kg of tubers, obtained from stock plants, grown in flats outdoors, were homogenized in ice cold methanol using a Waring Blendor. After filtration, the particulate residue was re-extracted with methanol by allowing it to stand for 24 hours at room temperature (27 ± 3 C). The two methanol extracts were combined and evaporated under vacuum at 30 C. The water insoluble material was removed by filtration. The aqueous solution was adjusted to pH 2.5 with dilute H_2SO_4 and extracted three times with 1/3 volume of diethyl ether (Reagent, Manufacturing Chemists). The ether extract was partitioned four times against a small volume of 1 M $NaHCO_3$ and water alternately. The alkaline extracts were combined and the pH adjusted to 2.5 with dilute H_2SO_4. This acidified aqueous phase was re-extracted three times with a small volume of ether. The acidic ether fraction was evaporated to dryness under vacuum.

Thin layer chromatography (TLC): The dried acidic ether fraction was dissolved in methanol and applied to TLC

plates coated with 0.25 mm silica gel (Merck). ABA was also spotted at both ends of the residue band to serve as markers. The chromatograms were developed in the following solvent systems:

(a) n-butanol: acetic acid: water (5:4:1)

(b) chloroform: ethyl acetate: acetic acid (60:40:5)

(c) isopropanol: ammonium hydroxide: water (100:14:6, thereafter referred to as PAW)

After development each Rf zone was scraped off the TLC plate and eluted twice with 2 ml of ether. The ether eluate was evaporated to dryness and used for bioassay. Silica gel from below the origin with an area equal to that of one zone was similarly eluted to serve as control.

The inhibitory zones (Rf 0.5 to 0.8) from the chromatogram developed in PAW were rechromatographed using the following solvent systems:

(a) chloroform: acetic acid (95:5)

(b) ether: ethyl acetate: acetic acid (50:5:2)

Each zone of the developed chromatogram was individually eluted and evaporated to dryness as described earlier, and used for bioassay.

The ABA marker spots on the chromatogram were identified by spraying with 5% concentrated H_2SO_4 in ethanol and heated to 110 C for 15 minutes. ABA appeared yellow and gave a green fluorescence under ultraviolet light (15).

Bioassay: (a) Wheat coleoptile elongation. The wheat coleoptile elongation test, described by Nitsch and Nitsch (55) for auxin bioassay, was modified for evaluation of inhibitor activity (18, 35). Wheat seeds were surface sterilized with 5% Clorox, for 5 minutes, washed, and soaked in water for 2 hours. They were germinated on moist filter paper in petri dishes at 27 C for 3 days in darkness. Four mm sections taken about 3 mm below the tip of the coleoptile were used for bioassay. Ten sections were placed in each 3 ml Lancer analyzer cup with the test solution. The test solution contained 0.5 ml extract buffered in 1 ml of 7×10^{-3}M K_2HPO_4 and 5×10^{-3}M citric acid at pH 5. The sections were measured after incubation for 20 hours in darkness at 27 C. Results were calculated as:

$$\text{Elongation as \% of control} = \frac{\text{change in length of treatment}}{\text{change in length of control}} \times 100$$

(b) Purple nutsedge buds. Lateral buds from the tubers were excised. Each bud was about 3 x 3 mm. Five buds were sprouted in 2 cm dishes containing 0.3 ml of the buffered aqueous extract (buffer as in the wheat coleoptile assay) with or without BA. BA was added to ensure sprouting of these excised buds. The dishes were placed in a moist plastic box in the growth chamber at 30 C with 12 hours photoperiod. Each treatment was replicated five to ten times. After 7 days the number of sprouted buds was recorded.

Gas-liquid chromatography (GLC): GLC procedures described by Davis et. al. (16) were followed. Inhibitors from Rf 0.5 to 0.8 of the chromatogram developed in PAW and those from Rf 0.5, 0.6, 0.7, 0.8, and 0.9 (each zone taken separately) from chromatograms developed in ether: ethyl acetate: acetic acid (50:5:2) were used for analysis.

Eluate obtained from the chromatogram was placed in a 3 ml test tube, dried over Na_2SO_4 and evaporated to dryness using a gentle stream of nitrogen. N, O-Bis-(trimethylsilyl) acetamide (BSA) (Pierce Chemical) was added to the residue at the rate of 0.2 ml BSA to 100 g tuber (fresh weight). A trimethylsilyl derivative of ABA (TMS-ABA) standard was also prepared. The test tube was capped and allowed to stand for at least 30 minutes before use.

GLC analysis were performed on a Varian 1800 Gas Chromatograph equipped with dual flame ionization detectors containing 1/8 in. x 5 ft. stainless steel column packed with 5% SE 30 on 80/100 mesh chromosorb W. The flow rate of carrier nitrogen gas and hydrogen was 25 ml/min., and air was 300 ml/min. The injector and detector temperatures were 200C and 260 C respectively. The column temperature was kept at 60 C for 6 minutes after which it was increased linearly by 12 C/min. until reaching a maximum of 240 C.

Effects of solvent residue: Since the purity of the solvents used in this study might be questionable both the

methanol and ether were evaporated to dryness and tested for any possible biological activity. These residues were also carried through similar TLC and GLC procedures, as described earlier, for bioassay and partial identification.

Chromogenic reagents: The following chromogenic reagents were used to detect phenolic compounds on the TLC chromatograms:

(a) diazotised p-nitro-aniline (DPNA): five ml of 0.5% p-nitro-aniline in 2 M HCl and 0.5 ml of 5% sodium nitrite in water were mixed while cooling in ice after which 15 ml of 20% sodium acetate in water was added (76).

(b) sucrose-HCl-ethanol: two g of sucrose was shaken in a mixture of 10 ml concentrated HCl and 90 ml of absolute ethanol. The suspension was sprayed on the chromatogram and heated in the oven for 30 to 60 seconds at 90 C (65).

(c) aqueous ferric chloride (2%) solution (61).

Results

Inhibitors from the tubers: Preliminary work showed that only the acidic ether extract from the tubers showed significant inhibitory activities. Therefore only this fraction was used in this study. As indicated by the wheat coleoptile assay (Fig. 1A, 1B & 1C) the ether extract showed many inhibitory zones. Although the ABA marker was present in one of these inhibitory zones, it was not the zone which

showed the greatest inhibition. In the PAW system (Fig. 1C) the inhibitory zones were from Rf 0.5 to 0.8. These were referred to as the inhibitor β complex by earlier workers (8, 32, 77), and will be likewise referred to as inhibitor β in this work.

Components of inhibitor β consisted of both inhibiting and non-inhibiting substances as tested by the wheat coleoptile assay. Several inhibitory zones were observed when inhibitor β was re-chromatographed in ether: ethyl acetate: acetic acid (50:5:2) and chloroform: acetic acid (95:5) solvents (Fig. 1D & 1E). The re-chromatographed inhibitor β tested by the excised bud assay (Fig. 1F) showed results similar to the wheat coleoptile assay (Fig. 1E). The only exception was that components in Rf 0.5, which inhibited wheat coleoptile elongation, did not inhibit bud sprouting. Four zones (Rf 0.6 to 0.9) inhibited bud sprouting and Rf 0.9 corresponded to the marker ABA (Fig. 1F).

A comparison of the activity of inhibitors from the tuber and that of ABA was shown in Figure 2. Fifty percent inhibition of wheat coleoptile elongation was obtained with inhibitor β from 3 g of tubers (Fig. 2A), with ABA at 0.5 ppm (Fig. 2B) and with Rf 0.9 from 6 g of tubers (Fig. 2C). Fifty percent inhibition of bud sprouting was obtained with Rf 0.9 from 3 g of tubers (Fig. 2D) and with ABA of 0.5 ppm (Fig. 2E).

FIGURE 1.--Bioassay of extracts from purple nutsedge tubers. Activity of extract from 15 g tubers (A, B, C) and the various components of inhibitor β from 10 g tubers (D, E) using the wheat coleoptile assay. Activity of the components of inhibitor β from 10 g tubers (with 1 ppm BA) as indicated by the excised bud assay (F). TLC was developed with the following solvent systems:

(a) butanol: acetic acid: water (5:4:1)

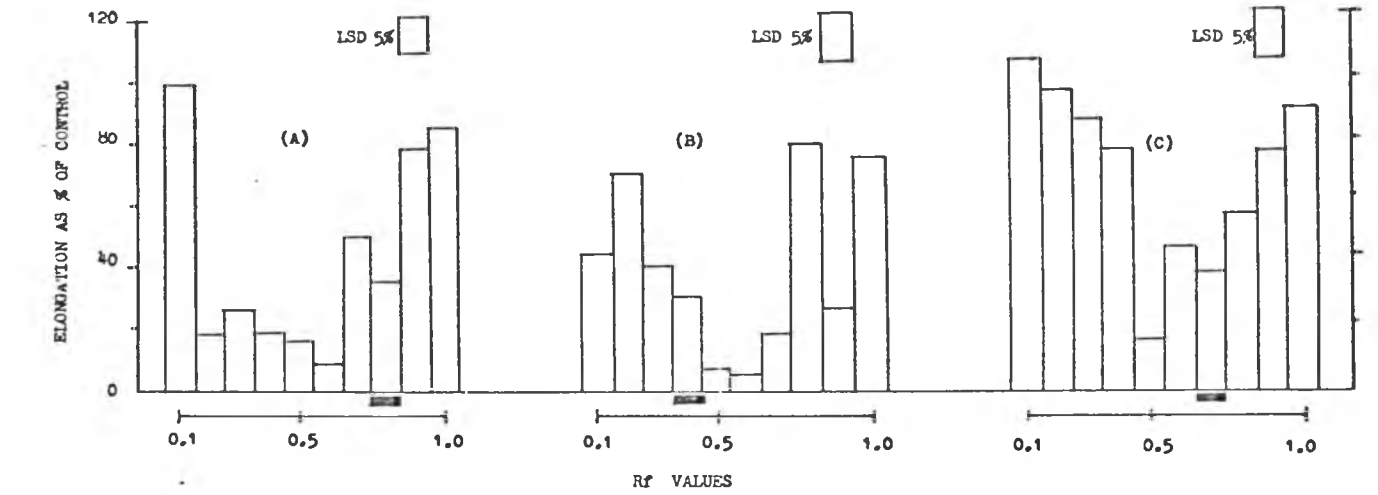
(b) chloroform: ethyl acetate: acetic acid (60:40:5)

(c) isopropanol: ammonium hydroxide: water (100:14:6)

(d) chloroform: acetic acid (95:5)

(e & f) ether: ethyl acetate: acetic acid (50:5:2)

C of histogram F indicates silica gel control. Solid rectangle on the x axis shows position of marker ABA as indicated by spraying with 5% H_2SO_4 in ethanol and heated in oven at 110 C.


120
80
40
0
ELONGATION AS % OF CONTROL
LSD 5%
(A)
(B)
(C)
0.1
0.5
1.0
Rf VALUES

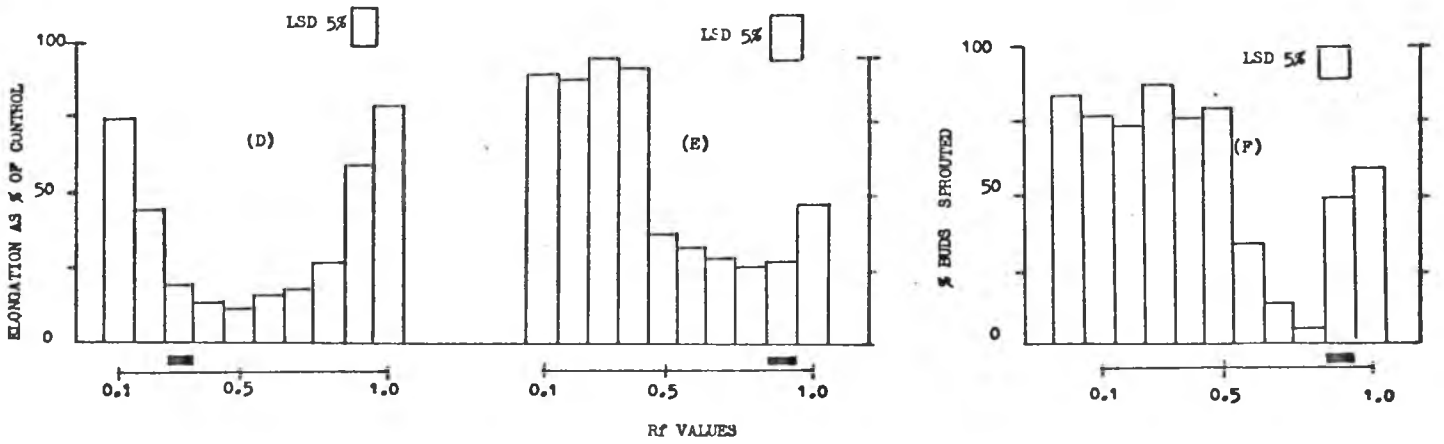
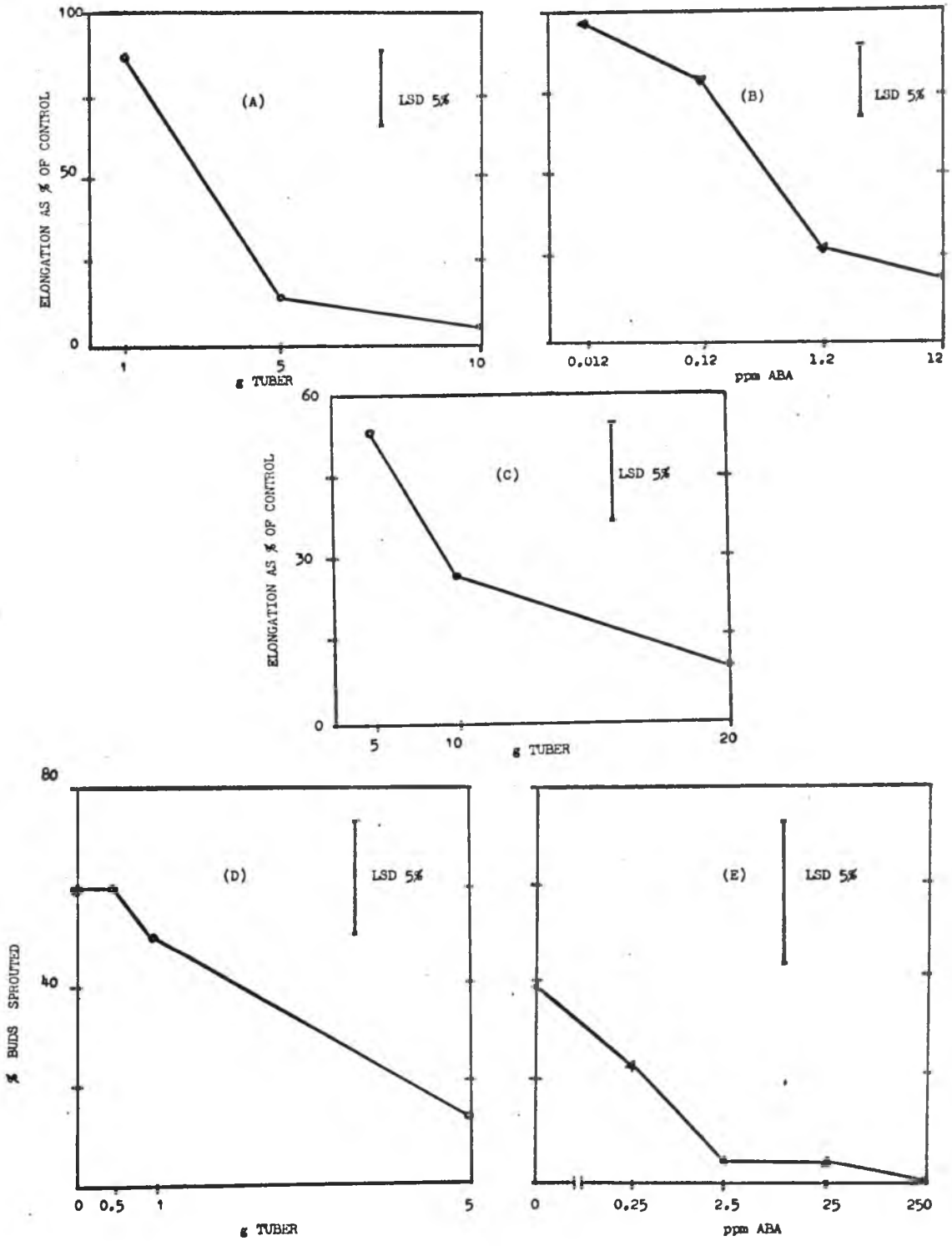

100
50
0
ELONGATION AS % OF CONTROL
LSD 5%
(D)
(E)
(F)
% BUDS SPROUTED
0.1
0.5
1.0
Rf VALUES

FIGURE 2.--Activities of inhibitors from purple nutsedge tubers and ABA. Activity of inhibitor β (A); ABA (B); and inhibitor from Rf 0.9 of chromatogram developed in ether: ethyl acetate: acetic acid (50:5:2) (C) using the wheat coleoptile assay. Activity of inhibitor from Rf 0.9 (D) and ABA (E) as indicated by the excised bud assay. Buds were treated with extract/ABA without BA.


(A)
LSD 5%
ELONGATION AS % OF CONTROL
100
50
0
1
5
10
g TUBER
(B)
LSD 5%
0,012
0,12
1.2
12
ppm ABA
(C)
LSD 5%
ELONGATION AS % OF CONTROL
60
30
0
5
10
20
g TUBER
(D)
LSD 5%
% BUDS SPROUTED
80
40
0
0.5
1
5
g TUBER
(E)
LSD 5%
0
0,25
2.5
25
250
ppm ABA

Inhibitor β from PAW significantly inhibited excised bud sprouting. Addition of 100 ppm BA with the extract relieved this sprout inhibition (Table 13). Treatment with inhibitor β from 5 g of tuber completely inhibited sprouting and BA did not reverse this inhibition.

TABLE 13.--Interactions of BA and inhibitor β from purple nutsedge tubers on sprouting of excised purple nutsedge buds (Buds were treated with buffered aqueous extract of inhibitor from various amounts of tubers with or without BA as indicated.)

Treatment	% Buds Sprouted	
	-BA	+ 100 ppm BA
Water control	80 a*	94 a
0.5 g tuber	40 b	85 a
1.0 g tuber	56 b	90 a
5.0 g tuber	0 c	0 c

*Means with the same letter are not significantly different at P = 0.05 (Duncan's Multiple Range test).

The reversal of sprout inhibition by BA was demonstrated in Table 14. Sprout inhibition induced by the various components of inhibitor β was relieved by treatment with BA. Inhibitors extracted from 10 g of tubers inhibited sprouting in spite of the presence of BA. A higher concentration of BA (100 ppm) did not reverse inhibition except

with the inhibitors from Rf 0.9 of which ABA is one of the components (Table 14). Bud sprouting was similarly inhibited by ABA and this ABA-induced inhibition was reversed with BA application. At a high ABA concentration (250 ppm) this reversal by BA could not be achieved with 100 ppm BA (Table 15).

Solvent residue: The impurities which might be present in the solvents used for extraction did not show any significant activity in either of the bioassay systems used (Table 16). The volume of the solvents used for this residue test was three times more than actually used for the tuber extraction.

TABLE 14.--Interactions of BA and the various components of inhibitor β re-chromatographed in ether: ethyl acetate: acetic acid (50:5:2) from Rf 0.5 to 0.8 PAW on sprouting of excised purple nutsedge buds (Buds were treated with buffered aqueous extract from various amounts of tubers with or without BA as indicated.)

Rf	% Buds Sprouted			
	5 g tubers	5 g tubers + BA 1 ppm	10 g tubers + BA 1 ppm	10 g tubers + BA 100 ppm
Water control	72 ad*	94 a	84 ad	90 a
0.6	30 b	66 ad	34 c	10 b
0.7	26 b	66 ad	13 b	0 e
0.8	15 b	86 ad	5 b	0 e
0.9	10 b	54 cd	50 c	80 ad

*Means with the same letter are not significantly different at P = 0.05 (Duncan's Multiple Range test).

TABLE 15.--Interactions of BA and ABA on sprouting of excised purple nutsedge buds (Buds were treated with BA, ABA, or their combinations as indicated.)

Treatments		% Buds Sprouted		
		BA (ppm)		
		0	1	100
ABA ppm	0	40 a*	100 d	-
	2.5	5 b	94 d	-
	25	5 b	0 c	90 d
	250	0 c	0 c	0 c

*Means with the same letter are not significantly different at P = 0.05 (Duncan's Multiple Range test).

TABLE 16.--A comparison of the activities of the various components of inhibitor β and solvent residues on wheat coleoptile elongation and sprouting of excised purple nutsedge buds (The volume of solvents used [methanol + ether] was equivalent to amount used to extract 30 g tubers. Separation was done on a TLC plate with ether: ethyl acetate: acetic acid [50:5:2] as solvent system. Data from effects of inhibitors on wheat coleoptile elongation and bud sprouting were derived from Fig. 1E and 1F respectively.)

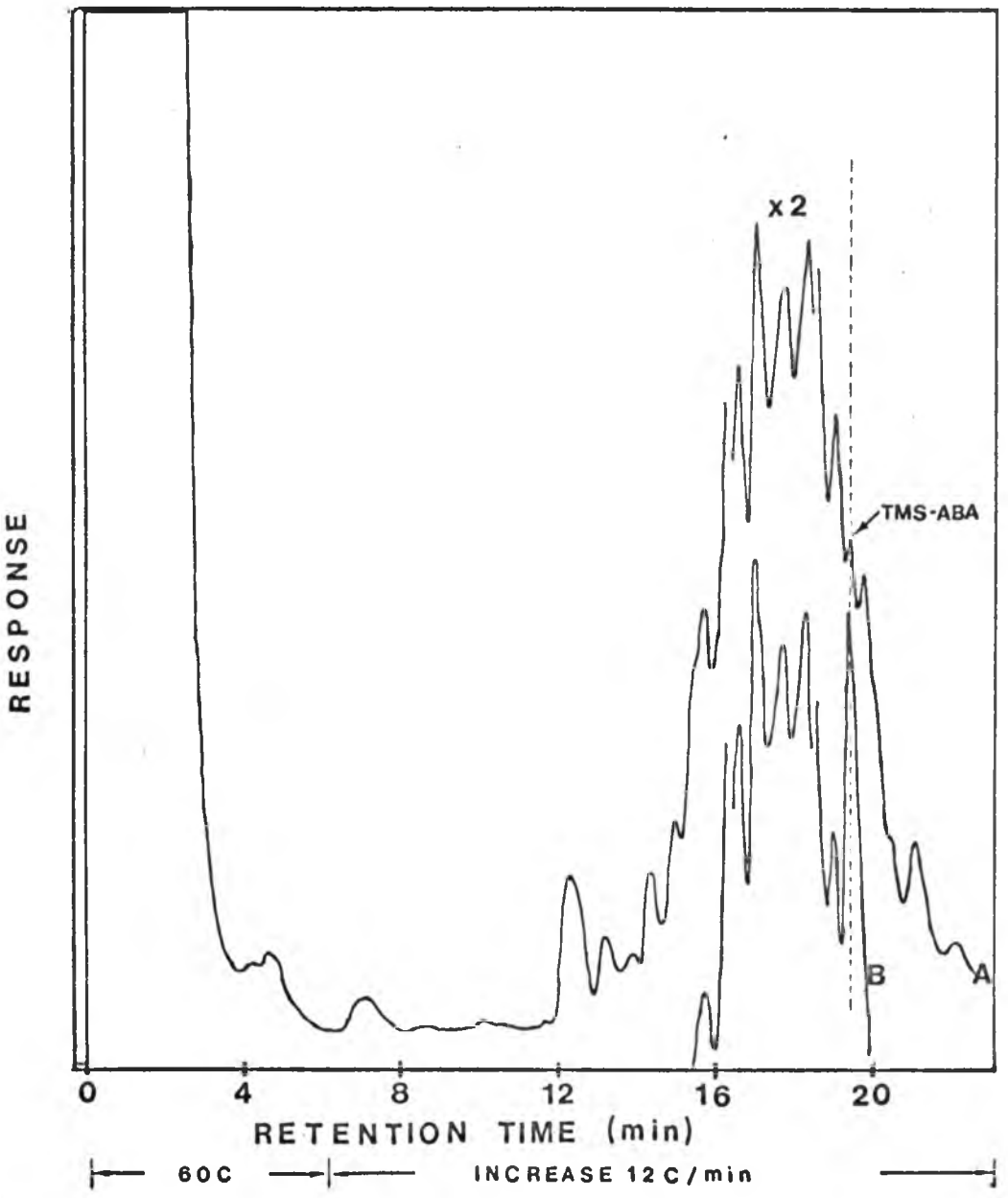
Rf	Wheat Coleoptile Elongation as % of Control		% Buds Sprouted	
	10 g Tuber	Solvent Residue	10 g Tuber	Solvent Residue
0.5	36 a*	103 b	80 c	86 c
0.6	32 a	103 b	34 df	100 c
0.7	28 a	113 b	13 de	80 c
0.8	26 a	98 b	5 e	74 cf
0.9	27 a	100 b	50 f	100 c

*Means within each paired column with the same letter are not significantly different at P = 0.05 (Duncan's Multiple Range test).

Partial identification of the inhibitors: The GLC analysis indicated that the BSA treated inhibitor β complex has a component which showed an identical retention time (20 min.) as the standard TMS-ABA. Upon further TLC purification of the inhibitor β in ether: ethyl acetate: acetic acid (50:5:2) this ABA-like substance eluted from Rf 0.9 of the chromatogram also contained ABA as suggested by the GLC (Fig. 3). When a mixture of the standard ABA and extract from Rf 0.9 was silylated and injected together both peaks coincided (Fig. 3). GLC analysis of other inhibitory zones (Rf 0.6, 0.7, and 0.8) did not show any ABA-like substance. Evidences based on the bioassay, TLC, and GLC results seemed to support that ABA was a constituent of inhibitor β.

Spraying a strip of TLC plate with DPNA gave a deep yellow coloration indicating that some of these substances possibly were phenolics. A high yellow intensity occurred between Rf 0.5 to 0.8. A chromatogram sprayed with sucrose-HCl-ethanol reagent showed a pink violet color at Rf 0.6 and violet at Rf 0.8, indicating that these might be di- and/or trihydroxy phenols. A light grey spot was obtained with Rf 0.7 when treated with 2% aqueous ferric chloride, again an indication of a phenolic compound.

FIGURE 3.--Gas-chromatograms of extract from purple nutsedge tubers. (A) Silylated inhibitor from Rf 0.9 of chromatogram developed in ether: ethyl acetate: acetic acid (50:5:2), and (B) silylated inhibitor from Rf 0.9 fortified with standard ABA.


RESPONSE
x2
TMS-ABA
B
A
0
4
8
12
16
20
RETENTION TIME (min)
60C
INCREASE 12 C / min

CHAPTER V

DISCUSSION AND CONCLUSIONS

Cytokinin has been found by many workers (11, 14, 21, 29, 45, 46, 49, 58, 59, 67, 68, 69, 81, 84, 85, 88) to break dormancy in seeds and resting organs. It was suggested that bud sprouting might be due to the action of cytokinin-like substances (49). Stimulation by cytokinins of sprouts from dormant buds in purple nutsedge tubers thus agrees with these reports. BA at concentrations 50 ppm and above significantly increased sprouting of purple nutsedge tubers but at 1 ppm BA was inhibitory (Table 1). This inhibitory effect of BA on sprouting seems puzzling and the author is unable to explain such effects.

Growth inhibitors were present in the tubers of purple nutsedge (Fig. 1). This is not unexpected since the occurrence of inhibitors in most plant tissues is a common phenomenon (48). There seemed to be more than one inhibitory substance in purple nutsedge tubers (Fig. 1). Based on the similarity of the Rf values and the solvent system (PAW) used, the inhibitors were referred to as inhibitor β complex (12, 32, 35, 77, 85). Rechromatography and bioassay showed that this inhibitor β complex had both inhibiting and non-inhibiting components. Using chromogenic reagents the components that inhibited coleoptile elongation and bud sprouting were found to be phenols. GLC analysis showed

the presence of an ABA-like substance among these inhibitors (Fig. 3).

The presence of inhibitor β, which was believed to be responsible for dormancy in potatoes was reported by various workers (12, 32, 77). The inhibitor β complex consists of various acidic and neutral substances, inhibiting and non-inhibiting components (9, 48, 81). Milborrow (51) identified ABA as the major inhibiting component of inhibitor β. Holst (35) also isolated ABA from the inhibitor of potatoes and found other growth inhibiting substances, including salicylic acid and some unidentified phenols, were present. Thus, results from the purple nutsedge tuber extract seemed to be consistent with the current view of natural plant inhibitors.

Although ABA has been suggested to play a role in the dormancy of purple nutsedge tubers (39), its occurrence in purple nutsedge has not been previously reported. Berger and Day (10) noted that there were many inhibitors present in the foliage and tubers of purple nutsedge but they cited salicylic acid as the major cause of tuber dormancy in spite of the fact that it was not found in the tubers. Other workers (22, 39) reported the presence of phenolic compounds in the foliage and subterranean structures of purple nutsedge. Although Friedman and Horowitz (22) thought that these phenols might be related to the physiology of growth inhibition of purple nutsedge, Jangaard et. al.

(39) failed to see any possible significant role played by these endogenous phenols.

Although this work established the presence of inhibitor β (including ABA and phenols) in purple nutsedge tubers, this is not sufficient evidence to substantiate its possible physiological role in causing tuber dormancy. The results from this study can afford a basis to hypothesize the possible relationship that might exist between inhibitor β or ABA and dormancy in purple nutsedge. ABA at 100 ppm inhibited sprouting of intact tubers but at lower ABA concentrations the number of sprouts produced was not significantly different from the control (Table 3). This number represented the sprouting of apical buds. Work by the author showed that 63% and 45% of the excised apical buds sprouted when treated with 10 and 50 ppm ABA respectively; while none of the excised lateral buds sprouted with similar treatments. One could thus expect that ABA would inhibit sprouting of excised lateral buds (Table 15) at a much lower concentration than intact apical buds (Table 3). Excised buds and buds on intact tubers may be expected to respond differently to applied growth substance due to various reasons, one of which is the variable permeability barrier. In both the experiments (Table 3 and 15), addition of BA relieved the ABA-induced inhibition, but BA was unable to relieve sprout inhibition of excised or intact buds at 100 ppm ABA.

The ability of BA to reverse the inhibitory effects caused by inhibitor β (Tables 13 and 14) and ABA (Tables 3 and 15) supported the hypothesis that the balance between inhibitors and promoters participates in controlling bud dormancy in purple nutsedge. Treatment of buds with inhibitor β or ABA caused the shift to favor inhibitor actions hence sprouting was inhibited. Addition of BA in the sprouting media permitted sprouting. One could hypothesize the possible role of BA in releasing dormancy is to antagonize the endogenous inhibitor action. Work of Khan (46), Sankhla and Sankhla (68), and Bewley and Fountain (11) showed that cytokinin was able to reverse ABA-induced germination inhibition of lettuce seeds. Similarly, Blumenthal-Goldschmidt and Rappaport (12) reported that sprout inhibition of excised potato eye induced by inhibitor β was reversed by GA. The role of hormones was reported to neutralize the effects of inhibitors in the tissues (44).

One hypothesis to explain dormancy in purple nutsedge is that the tubers are deficient in cytokinin and this leads to an imbalance of inhibitor-promoter complex, favoring the inhibitors. The inhibition effects could be due to ABA and/or a cumulative effect of the various components of inhibitor β. Addition of a cytokinin is necessary to restore a favorable balance of promoter for sprouting to occur. The suggested role of BA is to antagonize the inhibitor action.

The results of ethephon applications contradicted reports by earlier workers (38) where ethephon stimulated sprouting in nutsedge. This contradiction may be due to the differences in the type and source of tubers used. It has been suggested that the environment under which tubers are produced contributes significantly to their resultant dormancy (10) which may then reflect in differences of their response to applied growth substances.

The view that growth hormones participate in the control of rhizome orientation was reviewed by Phillips (60). Bendixen (7) reported that GA evoked erect growth in rhizomes of yellow nutsedge. The induction of diageotropic rhizomes by BA suggests a possible role in determining rhizome orientation.

It seems that the effect of BA in increasing the number of rhizomes (Table 12) was mainly to stimulate buds on the basal bulbs to form rhizomes. This phenomenon is analogous to the BA stimulation of bud sprouting in the tubers (Table 7). Buds on the tubers sprouted in darkness to form rhizomes, while in light plantlets resulted (Table 7). Further work of the author showed that basal bulbs sprouted to form rhizomes in darkness and plantlets in light.

The idea of stimulating buds on the tubers to sprout and form shoots may have a potential in preconditioning purple nutsedge for subsequent eradication. Enhanced

sprouting of purple nutsedge tubers can be achieved just as effectively in soil as in sand or petri dishes (Tables 1, 8 and 9). As shown in Table 9, an initial treatment of the soil with BA was effective in causing most buds on the tubers to sprout after which little or no sprouting occurred from subsequent BA treatment indicating that most of the viable buds present were exhausted. Assuming that a cytokinin-like substance is available for economical use, one question which needs to be answered is whether sprouts formed after such treatment would become normal plants. If so, what would be the possible effect of BA on subsequent plant growth? These experiments have shown that BA-treated tubers produced sprouts which grew normally. This means that buds can be induced by BA to sprout without simultaneously causing any additional undesirable effects on growth; namely, producing more plants or rhizomes. However, if BA were applied over an extended period of time and frequently enough, changes of growth habit and rhizome production might be expected (Table 12). Thus, a rapid breakdown of BA in the soil as observed in Table 10 seems desirable. Soil-applied cytokinin should have the sole function of inducing dormant buds on the tubers to sprout.

The response of purple nutsedge to cytokinins under field conditions has yet to be tested. BA and Kinetin are expensive and may not be of practical use in the field. It is hoped that a cheaper chemical exhibiting cytokinin-like

activity could be synthesized for economical use. Also there is a need to find a more efficient method of application of a cytokinin-like substance to induce maximal response. Foliar application of a cytokinin-like substance might be a cheaper, easier, and preferred method of preconditioning nutsedge than a soil application, however, preliminary studies by the author seems to indicate that such foliar applications would meet with limited success. Cytokinin needs to be in contact with the tubers to enhance sprouting (Tables 3 and 6). The penetration and translocation of cytokinin into the plant and tuber also appear to be a formidable problem.

The following are suggested areas for further research:

(1) A study on the role of light in determining rhizome or plantlet formation in purple nutsedge. Also the factors and agent(s) that cause the rhizome tip to swell into a tuber.

(2) A study on the interactions of BA and herbicides on tuber sprouting under field conditions.

(3) A study to correlate the levels of ABA or inhibitor β to dormancy in purple nutsedge. Also, the effects of the environment on endogenous inhibitor level.

CHAPTER VI

SUMMARY

A possible approach to effective purple nutsedge eradication is to induce all dormant buds on the tubers to sprout, after which a herbicide is used to destroy the foliage. Enhanced sprouting of purple nutsedge tubers by cytokinins was studied. The following results were obtained.

(1) Cytokinins (N-6 - benzyl adenine; kinetin; 6-beyzylamino-9-(tetra hydropyran-2-yl)-9H-purine) enhanced sprouting of purple nutsedge tubers when treated in petri dishes or when incorporated in the sand and soil. Sprouting was increased four to five-fold when compared to the control either in light or darkness.

(2) The continuous presence of BA during the sprouting period was necessary to give significant sprout stimulation. High temperatures (33 C day, 25 C night) increased sprouting, while low temperatures (24 C day, 17 C night) reduced it substantially. BA enhanced sprouting of both isolated tubers and tubers in intact tuber chains. Only tubers that were in contact with BA showed enhanced sprouting. Untreated tubers in the same chain with the treated tubers did not sprout.

(3) Growth of plants originating from tubers pretreated with BA did not differ significantly from the control.

Sustained foliar BA applications produced numerous plants with tuft-type growth habit, delayed flowering, and reduced number of inflorescence. Numerous short, diageotropic rhizomes were produced.

(4) Applications of abscisic acid and inhibitor β inhibited sprouting of the excised buds of purple nutsedge. BA released the inhibition imposed by these inhibitors.

(5) Inhibitor β complex consisted of many inhibitory substances of which ABA and phenolic substances were tentatively identified.

(6) One hypothesis to explain dormancy in purple nutsedge is that the dormant tubers are physiologically deficient in a cytokinin while inhibitor levels are high. Application of a cytokinin permits bud sprouting. The role of cytokinin is to antagonize the inhibitor actions.

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